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Specification as originally filed, with Application for Patent Serial No: **2,296,163**, on January 17, 2000, by **IMI INTERNATIONAL MEDICAL INNOVATIONS INC.**, assignee of Michael J. Evelegh, for "Test for Cancer".

Agent certificateur/Certifying Officer

February 5, 2003
Date

Canada

(CIPO 68)
04-09-02

OPIC CIPO

ABSTRACT OF THE DISCLOSURE

Colon-contacting semi-solid samples such as rectal mucus samples from human patients are analyzed for indications of abnormalities therein, such as evidence of cancerous or pre-cancerous conditions, by collecting the samples on a substantially white substrate, staining the samples with enzyme, e.g. galactose oxidase to develop color therefrom, and spectrophotometrically determining a defined color characteristic such as the hue angle, the chroma or saturation, or the lightness of the color so developed. It has been found that characteristics such as hue angle so determined correlate with cancerous abnormalities of the colon from which the mucus originated, allowing a non-subjective test and assessment to be conducted. Preferably, the substrate which is used is a substantially pure white, non-cellulosic, e.g. glass fiber material, to reduce background "noise" in the test procedure.

TEST FOR CANCER

FIELD OF THE INVENTION

This invention relates to medical test and screening procedures, and more specifically to tests fluids or semi-solids from human patients to obtain indications of possible cancerous or pre-cancerous conditions, and to kits useful in conducting such tests.

BACKGROUND OF THE INVENTION AND PRIOR ART

From U.S. patent 5,162,202, it is known to screen rectal mucous from human patients for detection of colorectal cancer and cancers of the large intestine. The mucous is collected on a membrane filter. A cellulose membrane filter is pre-prepared by impregnation with a solution of the enzyme galactose oxidase in a phosphate buffer, and then lyophilized. At the time of use, the cellulose membrane filter is moistened and then contacted with the membrane filter carrying the mucous sample, for 1-2 hours. Then the mucous bearing membrane filter is washed and reacted with basic fuchsin for 15 minutes, washed and dried. De-colorization of the fuchsin indicates the presence of carbohydrate markers of a cancerous or pre-cancerous condition in the mucous. Such a test is lengthy and tedious to perform, and does not have a high degree of sensitivity, so that it may give false negatives.

An improved rectal mucous test (RMT) is disclosed in U.S. Patent 5,348,860 Shamsuddin, issued September 20, 1994. In this procedure, the mucous sample is collected and immobilized on a membrane filter, and is treated with galactose oxidase to effect oxidation of any vicinal galactose moieties in the sample to vicinal aldehyde moieties. These are visualized with Schiff's Reagent. This is a more rapid procedure. Samples which test negative by this procedure can be further oxidized with periodic acid and then visualized with Schiff's Reagent, so as to reduce the chances of obtaining false negative results.

A continuing problem with known RMTs is that the staining results need to be visually examined and appraised. Whilst such examinations are adequate as a preliminary, subjective assessment for the presence of absence of cancer markers, they are qualitative only. They do not give reliable quantitative information about the amounts and concentrations of markers which have been found, and which could give indications of the state of progression of the cancer condition, if present. Moreover, the medium on which the samples are developed, normally a cellulose membrane, such as filter paper, may itself contain compounds capable of participating in the color development reactions. This can give a "background" which complicates the interpretation of the test results, and reduces its sensitivity. This requires a trained individual skilled in the interpretation of the test result.

It is an object of the present invention to provide a novel test of rectal mucous and other secretions, liquid and semi-solid, including stool, and mixtures thereof, and a kit for use therein, which overcomes or at least significantly reduces one or more of the above disadvantages. In the following description, the term "colon-contacting semi-solids" is used to denote mucus, stool and other liquids or seni-solids obtained from the rectum or colon of a patient, and mixtures thereof, which provide the analyzable material for use in the process of the present invention.

SUMMARY OF THE INVENTION

In the process of the present invention, a portable spectrophotometer is used to view and analyze the colors developed on enzymatic oxidation of carbohydrate markers present in colon-contacting semi-solid samples from patients. Certain specific parameters detectable with a spectrophotometer, but not previously considered in this connection, are examined and analyzed, to give a diagnosis of enhanced sensitivity and specificity. The process of the invention measures color at various wavelengths, and determines one or more defined characteristics of the measured color such as the hue angle, the chroma or

saturation, or the lightness, from which it has been surprisingly found that much valuable information concerning the presence or absence of progressive diseases such as cancer, and its state, can be derived.

Another aspect of the present invention relates to the medium on which the colon-contacting semi-solid sample is reacted with the enzyme and color developed. For this purpose, the present invention utilizes a membrane of a specific glass fiber material with low background staining and being pure white in color and which has a porous, "pebbled" surface. Such material essentially eliminates "background", in the sense of developing colors from residues other than those originating in the sample under test. Further, it allows the sample to spread over a greater surface area, due to its surface nature, thereby exposing more of the carbohydrate residues from the sample to color development reactions and so increasing the sensitivity of the test. The pure white color of the membrane reduces color "noise" when reading the test result. Conveniently the glass fibre membrane retains the colon-contacting semi-solid sample during processing.

Thus according to the present invention, in one aspect, there is provided a process of diagnosing rectal colon-contacting semi-solid samples for evidence of abnormalities in the source patient, which comprises collecting a colon-contacting semi-solid sample from a patient, depositing at least a portion of the sample on a generally white substrate, staining the sample on the substrate with galactose oxidase, color developing the stained sample with Schiff's reagent, determining a defined color characteristic of the developed color of the sample by spectrophotometry, and classifying the sample as normal or abnormal according to the value of the defined color characteristic so obtained.

According to another aspect of the invention, there is provided a kit for analysis of semi-solid colon-contacting samples obtained from human patients to diagnose for the presence or absence of rectal abnormalities in the patient, comprising;

a generally white, non-cellulosic substrate for receiving the sample;

a source of galactose oxidase;

a source of Schiff's reagent;

and a portable reflectance spectrophotometer capable of determining and reporting a defined color characteristic selected from hue angle, chroma or saturation, and lightness from stained samples on said substrate.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Suitable spectrophotometers for use in the present invention are portable, reflectance-based, and give accurate measurements of color characteristics such as hue angles, lightness and chroma or saturation, when the incident light of the spectrophotometer is reflected back from the stained sample to the instrument's receiver. They are commercially available. A specific example of a suitable such instrument is that marketed by X-Rite, Grand Falls, Michigan, U.S.A as "Model CA22 Spectrophotometer. It is supplied with appropriate software so that it can be connected to a computer to give an accurate read-out of the hue angle of the stained sample under test. The spectrophotometer receives reflectances over the approximate wavelength 400-700 nm, i.e. over most of the visible light spectrum, suitably over about 20 nm intervals.

It is known that color may be defined and expressed in terms of hue angle. The concept of "hue angle" is defined and discussed in standard textbooks such as "Principles of Color Technology," by Fred W. Billmeyer and Max Saltzman, published by John Wiley and Sons (see particularly Chapters 1 and 2), incorporated herein by reference. "Hue" is the color or shade of a specimen independent of its brightness or intensity, and "hue angle" of a color or shade is the definition of its reflectance wavelength by angular position with reference to a standard three dimensional ellipsoidal continuum plot of the entire spectrum of visible light. The visible light (color) continuum is represented on an angular scale from 0 to 360°, and the angular values as read by the reflectance spectrophotometer are transformed into linearized form to give the transformed "hue angle" used in the process of the present invention.

It has been surprisingly found, in accordance with the invention, that the presence of bowel pathology can be determined by determining the hue angle or other defined color characteristic mentioned above, of the stained colon-contacting semi-solid such as rectal mucus. Thus, individuals with polyps give rectal mucus samples which after staining and color developing as described, have higher hue angle numbers than those with normal bowels, and those with colorectal cancer give samples developing hue angles higher than those with polyps. Thus the hue angle or other defined color characteristic of the stained sample can be used to differentiate individuals with pre-cancerous or cancerous lesions of the bowel from those without such lesions. More specifically, samples from cancerous lesions have been found to give hue angles generally in the range 375-425°, the top quartile of measurement from clinical samples. Further, because the test result is interpreted by the portable spectrophotometer, there is no requirement that the test results be produced by a skilled, trained individual.

A significant aspect of the preferred embodiments of the present invention is the use of a porous glass fiber membrane on which the sample is oxidized and color developed. Such as glass fiber material is essentially free from stain-producing residues, so that it presents no residues which will undergo enzymatic oxidation so as to participate in the subsequent color developing reaction. Accordingly, background color development likely to confuse or interfere with the diagnostic tests, is effectively eliminated. Moreover, the membrane is essentially pure white in color, further reducing background "noise" against which the results are read.

A further characteristic of the glass fiber membrane used in this aspect of the invention is its surface porosity, which allows additional spreading of the mucus sample thereon so as to expose additional carbohydrate markers in the sample to participate in the oxidation and color development reactions, with consequent improved sensitivity of test method.

A specific, preferred example of a glass fiber porous membrane for

use in the present invention is that available commercially from Whatman Inc, Laboratory Division under the designation "Whatman 934-AH Glass Microfiber Filter", a borosilicate glass filter medium having high loading capacity and high retention efficiency at high flow rates. It is recommended for use in cell harvesting and liquid scintillation counting techniques. This is, however, exemplary only, and other, substantially pure white glass fibre, carbohydrate free substrates of surface porosity suitable to effect surface spreading of the colon-contacting semi-solid sample can also be used.

In a specific procedure using the present invention, first the sample to be tested is obtained from the patient. Lubricant is supplied to the gloved finger of the operator. The finger is inserted into the rectum of the patient and rotated 360° to obtain a representative sample of colon-contacting semi-solid such as rectal mucus. The finger is removed from the rectum and the sample is smeared onto the surface of the white membrane filter described above, mounted on an RMT card with appropriate covering, protection and identification, and the card is sent to the laboratory for testing.

At the laboratory, the backing is removed from the RMT device, and one drop of standard galactose oxidase solution is added to the test card. Incubation proceeds, in the standard way, for 5 minutes. Then the card is dipped into PBS and transferred to Schiff's reagent for 5 minutes. The color is then developed by transferring the card through 3 water rinses for 1 minute each rinse. The card is blotted dry, and the RMT score is determined by reading the hue angle with the portable spectrophotometer, of the type previously described.

From the values of hue angle simply read out of the spectrophotometer in this way, the operator can, without any subjective interpretation, determine whether the sample originates with a patient having healthy, normal colon, pre-cancerous colon or cancerous colon, with greatly reduced chances of false positive or false negative readings, as compared with prior diagnostic methods. The same standard collection, staining, incubation and

color development steps are taken, and the same standard reagents are used, so that the new procedure according to the invention can be adopted by established diagnostic laboratories with minimum disruption and economic expenditure.

WHAT IS CLAIMED IS:

1. A process of diagnosing colon-contacting semi-solid samples for evidence of abnormalities in the source patient, which comprises collecting a colon-contacting semi-solid sample from a patient, depositing at least a portion of the sample on a generally white substrate, staining the sample on the substrate with galactose oxidase, color developing the stained sample with Schiff's reagent, determining at least one defined color characteristic selected from hue angle, chroma or saturation, and lightness of the developed color of the sample by spectrophotometry, and classifying the sample as normal or abnormal according to the value of the color characteristic so obtained.

2. A process of analyzing a colon-contacting semi-solid sample collected from a human patient and deposited on a generally white substrate, which comprises developing color from said sample by enzyme reaction, determining at least one defined color characteristic selected from hue angle, chroma or saturation, and lightness, of the color so developed, and classifying the sample as normal or abnormal according to the defined characteristic of the color so developed.

3. The method of claim 2 wherein the defined color characteristic is the hue angle, and the hue angle is determined spetrophotometrically.

4. The process of claim 1 or claim 3 wherein the substrate is non-cellulosic.

5. The process of claim 4 wherein the substrate is glass fiber.

6. The process of claim 5 wherein the substrate is substantially pure white.

7. The process of any preceding claim wherein the sample is

predominantly a rectal mucus sample.

8. A kit for analysis of colon-contacting semi-solid samples obtained from human patients to diagnose for the presence or absence of rectal abnormalities in the patient, comprising;

a generally white, non-cellulosic substrate for receiving the sample;

a source of galactose oxidase;

a source of Schiff's reagent;

and a portable reflectance spectrophotometer capable of determining and reporting at least one defined color characteristic selected from hue angle, chroma or saturation, and lightness, from stained samples on said substrate.

9. A kit according to claim 8 wherein the substrate is glass fiber.